REMARKS

Entry of the foregoing pursuant to and consistent with 37 C.F.R. §§ 1.821-1.825 is respectfully requested in light of the remarks which follow. No new matter is believed to have been added by way of this Amendment.

The specification purportedly lacks sequence identifiers (*e.g.*, the primers on page 44 and the nucleotides in Figure 7). Applicants note that these sequences (SEQ ID NOS. 1 and 2 for the primers and SEQ ID NOS. 5 and 6 for Figure 7) are present in the sequence listing already on file. The sequences were identified in the August 29, 2001 Reply to Notice to File Missing Parts of Nonprovisional Application (dated June 29, 2001). A marked-up copy of the pages of the specification identifying the appropriate sequence identifiers was filed on August 29, 2001.

Paragraph number 7 of the Notice to Comply indicates that "if these sequences are present in the sequence listing already in the file, it would be remedial to simply amend the specification to include the appropriate sequence identifiers (*i.e.*, SEQ ID NO:) at the appropriate points". Thus, Both a marked-up copy of the pages of the specification and a clean copy of the pages of the specification, wherein the appropriate sequence identifiers are indicated, is filed herewith. Applicants submit that a substitute sequence listing is not required because the sequences are present in the sequence listing already on file.
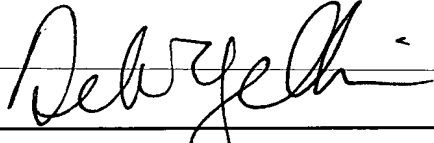
C O N C L U S I O N

Applicants submit that this application is fully in condition for examination

according to U.S.C. §§ 131 and 132.

In the event that there are any questions relating to this Reply, or the application in

general, it would be appreciated if the Examiner would telephone the undersigned attorney

concerning such questions so that prosecution of this application may be expedited.

Respectfully submitted,

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By: _____

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Date: February 4, 2003

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Page 33, Paragraph Beginning at Line 30

Figure 7 illustrates the adenoviral origin of replication. The binding sites for the

viral pTP-pol complex and cellular factors NFl and Oct-1 are indicated for the Ad5

sequence (SEQ. ID NO.: 5). The sequence (SEQ ID NO.: 6) from BAV3 is shown aligned

with the sequence from Ad5.

Page 44, Paragraph Beginning at line 1

generated by homologous recombination between a transfer plasmid named pTG 14866

(treated with Xbal and calf intestinal phosphatase, CIP) and a plasmid containing a

modified version of the hFIX gene (pTG 14869, digested with Sf1). pTG 14866 contains

the AdS cis-acting elements plus promoter sequences and the 3' end of the hFIX gene,

separated by a unique XbaI site and was constructed as follows. pTG 8343, containing the

5' end of the AdS genome with a deletion in the El region is used as a source for the 5' end

of the minimal vector. It is digested with AatII-BglII and overhanging end are made blunt

using Klenow enzyme. The Ad5 3' ITR is isolated from plasmid pTG 5670 which contains

the ITR, flanked by a PacI site on one end and a multiple cloning site on the other. The

ITR is excised as a BglII fragment, treated with Klenow enzyme and ligated into pTG 8343

prepared as described above. The resulting plasmid, pTG 14138, contains the 5' end from

Ad5 from nt 1 to 454, a MCS and the 3' ITR from nt 35828-35938. 5'flanking sequences

from the hFIX gene are isolated from pTG 3960 (described in Jallat et al.) as a 1.4 kb

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fragment by digestion with PstI and HindIII, and introduced in PstI-HindhII digested pTG

14138 by ligation resulting in pTG 14159. Next, 3' flanking sequences from the hFIX gene

are isolated from pTG 3960 as a PCR fragment. To this end, primers OTG 2070 (SEQ ID

NO.: 1) 5'-AGAGCTTGTATGGTTATGGAGG-3') and OTG 1224 (SEQ ID NO.: 2) (5'-

CACGATACTCGATGCAAGAC-3') are used to amplify a 1.6 kb fragment which is

introduced by ligation in pTG 14159 which was linearized with XbaI, and made blunt using

Klenow enzyme. This finally resulted in transfer plasmid pTG 14866. Before hFIX

sequences were introduced in this transfer plasmid, we removed 5 consecutive PacI sites

present in intron D of the hFIX gene in plasmid pTG 3960. This is necessary, because we

will use PacI to excise the final minimal vector from the plasmid vector backbone. If PacI

sites are present in the minimal vector sequences, this will result in fragmentation of the

minimal vector genome. In order to

Paragraph beginning at page 45, line 9

pTG 14872:   In parallel with pTG 14342, a minimal vector containing hFIX as stuffer

DNA was constructed. Instead of using transfer plasmid 14866 containing

the 5 'and 3' flanking sequences of the hFIX gene, pTG 14868 was used. It

contains part of intron A downstream of the adenoviral 5' hR and

encapsidation signal instead of sequences upstream of the hFIX promoter.

The construction was started with pTG 14138, which contains the Ad5 cis-

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acting sequences separated by a MCS (see above) and which was linearized

in the MCS by digestion with HindIII followed by treatment with Klenow.

Into this vector was ligated a 1.1 kb Seal fragment from pTG 3960

containing part of intron A from the hFIX gene. pTG 14160, with the insert

in the sense orientation was thus obtained. Next a unique BamHI site in the

remainder of the MCS between the AdS packaging signal and the intron A

insert was destroyed, while at the same time a NotI site was introduced. This

was done by digesting pTG 14160 with BamHI and ligating oligonucleotide

OTG 3497 treated with polynucleotide kinase in the BamHl site. OTG 3497

(SEQ ID NO.: 3) (5'-GATCGCGGCCGC-3') is capable of forming a NotI

site flanked by BamHI compatible cohesive ends by auto-hybridization. The

resulting plasmid, pTG 14867, is used to introduce the 3' flanking region of

the hFIX gene needed for homologous recombination in the next step. The 3'

flanking sequence is isolated as an XbaI-KpnI fragment from pTG 14866 and

ligated into XbaIKpnI digested pTG 14867. The resulting transfer plasmid

pTG 14868 is treated with XhaI and Sf1, which allows for the

Paragraph beginning at page 46, line 6

pTG 14742:   We have chosen to introduce expression cassettes in the unique PmeI site of

the empty minimal vector pTG 14872. The PmeI site is located in intron D.

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In order to facilitate introduction of foreign cassettes, we have sub-cloned a

fragment containing the PmeI site in a small vector, flanked by NotI sites.

Introduction of the expression cassette in the PmeI site can be done in this

vector. After excision of the expression cassette with flanking hFIX

sequences by the action of NotI endonuclease, the cassette can be introduced

by homologous recombination in the empty minimal vector which has been

linearized with PmeI. The first step in the construction of the transfer

plasmid consisted in introducing an EcoRI site flanked by NotI sites in

phagemid pBluescript II SK$^+$ which was purchased from Stratagene. The

phagemid was digested with PvuII and CIP, thus removing a *445* bp

fragment containing the MCS flanked by bacteriophage 13 and T7 promoter

sequences. The restriction enzyme recognition sites were introduced by

ligation using a polynucleotide kinase treated, self-complementary

oligonucleotide (OTG 1 2753 (SEQ ID NO.: 4), 5'-

CAGGCGGCCGCGAATTCGCGGCCGCCTG-3'). The resulting plasmid is

named pTG 15154 and can be used directly to introduce a hFIX fragment.

This was done by cutting pTG 15154 with EcoRI and removing terminal *5'*

phosphate groups with CIP followed by ligation involving a 3.0 kb MfeI

fragment from pTG 3960 containing the PmeI site. The resulting transfer

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plasmid pTG 14742 can be used directly for the introduction of foreign DNA sequences.